BOX PCT

Preliminary Amendment 5A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: A.G. Uitterlinden et al. Attorney Docket No.: KILS117128

Application No.: 09/786,991

Filed: March 9, 2001

Title: METHOD FOR DETERMINING THE SUSCEPTIBILITY TO BONE DAMAGE BY SCREENING POLYMORPHISMS IN THE VITAMIN D RECEPTOR

PRELIMINARY AMENDMENT

Seattle, Washington 98101

TO THE COMMISSIONER FOR PATENTS:

Please enter the following Preliminary Amendment into the above-referenced patent application.

In the Specification:

On page 1, immediately after the title, please enter the following:

RELATED APPLICATIONS

The present application is the U.S. national phase of PCT/EP99/07719, filed September 10, 1999, which claims benefit of priority from British Patent Application No. GB9819769.2, filed on September 10, 1998, the benefit of priority of which applications is claimed under 35 U.S.C. § 119 and 120.

Amend page 9, lines 18 and 19, as follows:

1. 5'-CAACCAAGACTACAAGTACCGCGTCAGTGA-3' (SEQ ID NO:1) and/or
2. 5'-GCAACTCCTCATGGCTGAGGTCTC-3' (SEQ ID NO:2)

Amend page 9, lines 26 and 27, as follows:

1. 5'-TAACTTCTGGACTATTTGCGGACTTTTTGG-3' (SEQ ID NO:3) and/or
2. 5'-GTCCAGCCCTCATCCTGGCC-3' (SEQ ID NO:4)

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In the Claims:

Amend Claims 6, 7, 9, 11, 13 and 15, cancel Claim 5, and add new Claims 27-30, as shown below.

1. A method of determining susceptibility to bone fracture in a subject, said method comprising analysing genetic material of a subject to determine the presence of the baT haplotype of the vitamin D receptor gene, wherein said haplotype is associated with risk of bone fracture.

2. A method of determining susceptibility to bone damage according to claim 1, said method comprising analysing the genetic material of a subject to determine which of the B/b, A/a and T/t alleles of the *Bsm*I, *Apa*I and *Taq*I sites of the vitamin D receptor are present.

3. A method of determining susceptibility to bone fracture according to claim 1 or claim 2, said method further comprising analysing the genetic material of a subject to determine which allele of the collagen Iα1 gene is present.

4. A method of determining susceptibility to bone fracture according to claim 3, said method comprising determining the presence of a G to T polymorphism at the *Sp*1 site of the collagen αI1 gene.

Sub B5

6. (Amended) A method of determining susceptibility to bone fracture according to claim 3, said method comprising determining the copy number of the B/b, A/a or T/t alleles of the vitamin D receptor gene and/or the S/s allele of the collagen Iα1 gene.

7. (Amended) A method according to claim 3 further comprising determining whether the allele(s) or haplotypes of the vitamin D receptor gene or collagen Iα1 gene present is/are associated with risk of bone fracture.

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8. A method according to claim 6 comprising comparing the allele(s) present in the genetic material of the subject with genotypes of the vitamin D receptor or collagen Iα1 genes having known degrees of risk of bone fracture.

Q5 9. (Amended) A method according to claim 3, further comprising determining calcium levels in a subject.

10. A method according to claim 9 wherein daily calcium intake is measured.

Q6 11. (Amended) A method according to claim 1, wherein said method is performed *in vitro*.

12. A method according to claim 11, wherein said method is performed on blood, or tissue samples of a subject.

Sub B7 13. (Amended) A method according to claim 1, further comprising treating the subject to reduce the risk of bone fracture.

14. A method according to claim 13, wherein suitable treatments include modifications to lifestyle, regular exercise, changes in diet or pharmaceutical preparations.

Q8 15. (Amended) A method according to claim 1, wherein the subject is a mammal.

16. A method according to claim 15, wherein the subject is a human.

17. A method according to claim 15 or 16, wherein the subject is a female.

18. A method of predicting response of a subject to treatment, said method comprising analysing genetic material of a subject to determine the presence of the baT haplotype of the vitamin D receptor gene, wherein said haplotype is associated with risk of bone fracture.

19. A method according to claim 18, further comprising determining which allele(s) of the collagen Iα1 gene is/are present.

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20. A method according to claim 18, wherein said subject is diagnosed as being susceptible to bone fracture.

21. A method according to claims 18 or 19 further comprising administering the appropriate treatment.

22. Use of a kit to determine susceptibility to bone fracture in a subject, said kit comprising (i) one or more nucleic acid primer molecules for amplification of a portion of the vitamin D receptor gene, and (ii) means for determining whether the baT haplotype of said gene is present.

23. Use of a kit according to claim 22, further comprising (i) one or more nucleic acid primer molecules for amplification of a portion of the collagen Iα1 gene and (ii) means for determining which allele of said gene is present.

24. A kit for determining susceptibility to bone fracture in a subject, said kit comprising (i) one or more nucleic acid primer molecules for amplification of a portion of the vitamin D receptor gene, (ii) means for determining whether the baT haplotype of said gene is present; and (iii) means for indicating correlation between said allele(s) and risk of bone fracture.

25. A kit according to claim 24, said kit further comprising (i) one or more nucleic acid primer molecules for amplification of a portion of the collagen Iα1 gene and (ii) means for determining which allele of said gene is present.

26. A kit according to claim 24 or claim 25, said kit comprising DNA control samples, for comparison with DNA sequences of a subject.

Sub B11

27. (New) A method according to claim 1, wherein the haplotype may be determined by amplification of a relevant portion of the vitamin D receptor gene, followed by restriction enzyme digestion; or any other technique suitable for determining the genotype of a subject.

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28. (New) A method according to claim 2, wherein the haplotype may be determined by amplification of a relevant portion of the vitamin D receptor gene, followed by restriction enzyme digestion; or any other technique suitable for determining the genotype of a subject.

29. (New) A method according to claim 3, wherein the haplotype may be determined by amplification of a relevant portion of the vitamin D receptor gene or collagen Iα1 gene, followed by restriction enzyme digestion; or any other technique suitable for determining the genotype of a subject.

30. (New) A method according to claim 4, wherein the haplotype may be determined by amplification of a relevant portion of the vitamin D receptor gene or collagen Iα1 gene, followed by restriction enzyme digestion; or any other technique suitable for determining the genotype of a subject.

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## REMARKS

This preliminary amendment conforms the claim dependencies of the above-referenced patent application to U.S. practice. The Examiner is requested to enter the foregoing claim amendments prior to examining the application.

Enclosed is a certified copy of the following application for which a claim of priority under 35 U.S.C. § 119 has been made:

| Country | No. | Filed |
|---|---|---|
| Great Britain | GB9819769.2 | 10 September 1998 |

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the U.S. Postal Service in a sealed envelope as first class mail with postage thereon fully prepaid addressed to: Commissioner for Patents, Washington, D.C. 20231, on the below date.

Date: 5/7/01

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